CHAPTER 21

REPRODUCTIVE BIOLOGY

Sex Change in Cycads—Cases, Causes, and Chemistry

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Abstract

Thirty-three cases of sex reversals in six cycad genera and seventeen species are summarized. Unidirectional male-to-female and female-to-male changes have been observed and are typically associated with gross physical damage or severe environmental changes. We propose that sex expression in dioecious plants is governed by the presence or absence of methylation of DNA cytosine residues on genes that regulate production of gametes and/or sex hormones.

Resumen

Se resumen treinta y tres casos de reversión sexual en seis géneros de cícadas y 17 especies. Se han observado cambios unidireccionales masculino-femenino y *vice versa* que son típicamente asociados con daños físicos fuertes o cambios severos de ambiente. Proponemos que la expresión del sexo en plantas dioicas está gobernado por la presencia o ausencia de la metilación de residuos de ADN citosina sobre los genes que regulan la producción de gametos y/o hormonas sexuales.

Introduction

Sexual behavior in plants and animals is probably the most discussed, researched, and misunderstood of all biological processes. The eighteenth century concept of sex being genetically determined in organisms at the time of fertilization, and immutable thereafter, still finds support among some scientists. But sex changes are now known to be so widespread throughout the plant and animal kingdom that biologists accept that many organisms inherently have both male and female genetic propensity and that environmental influences can result in sex changes. In this paper, we discuss all known cases of sex reversals in cycads and attempt to find underlying causes for these changes. Furthermore, we propose a mechanism by which the sex expression in dioecious plants is controlled.

Known Cases of Sex Change in Cycads

CHRONOLOGICAL RECORD OF DOCUMENTED CASES OF SEX CHANGE IN CYCADS

The earliest reference to sex change in cycads is that mentioned by Schuster (1932), who tells of a *Cycas revoluta* plant, which was longitudinally bisected (one wonders why), with both halves surviving but one being male and the other female. Charles Chamberlain (1935), although adamant on the absolute dioeciousness of cycads, records two incidents of sex reversal: (1) a cultivated plant of *C. revoluta* in Australia that changed from female to male and (2) a bud from a female specimen of *Cycas circinalis* (more probably *Cycas rumphii*) from a Chicago conservatory that later produced a male cone. Menniger (1967), in his book *Fantastic Trees*, also mentions two incidents of cycad sex reversal: a female specimen of *C. circinalis* (again, more probably *C. rumphii*) that changed to a male after being physically damaged and a male of the same species that produced a female cone after severe frost exposure.

Van Wyk and Claassen (1981) give a detailed account of a particular sex reversal that relates to one of several specimens of *Encephalartos umbeluziensis* growing in Dr. Claassen's garden in Pretoria, South Africa. The specimen in question produced a male cone in 1970 but a female cone in 1979 and thereafter. This plant was in a more exposed area, and the authors speculated that a freak cold weather spell in 1972 may have initiated the sex change.

Encephalartos, the journal of the Cycad Society of South Africa, has publicized numerous incidents of sex change in garden-grown cycads. A *C. revoluta* changed from male to female after being transplanted (Kemp, 1985). A female *Zamia* plant reportedly produced a side branch bearing a male cone (Osborne, 1985). A specimen of *Encephalartos latifrons* bore two successive crops of female cones with viable seeds after being transplanted in 1970 but then produced male cones for two years after a severe drought in 1983 in the eastern Cape (Osborne, 1988). A seed-grown *Stangeria eriopus* plant that had produced female cones for several years produced a side crown with a male cone (Anonymous, 1989). An *Encephalartos villosus* plant subjected to particularly dry conditions changed from female to male (Osborne, 1990). A female plant of the same species, which produced a cone and viable seeds in 1994, yielded an aborted cone in 1997 after being transplanted but then produced only male cones after 1998.

Osborne (1990) gave an account of a female specimen of *Encephalartos senticosus* that regularly produced cones and viable seeds. This plant produced nine basal suckers that were removed and rooted, and three of these suckers were subsequently monitored; one produced female cones, one produced male cones for three successive years, and the third did not produce cones at all. A female specimen of *Encephalartos middelburgensis*, which gave regular seeds crops in a garden in Durban, South Africa, produced six male cones one year (Fig. 21-1) (Osborne, 1991). A female plant of *C. revoluta* in a garden in Genoa, Italy, produced a male cone one year (Osborne, 1994). Four of 19 male *Zamia vazquezii* specimens at the Lowveld National Botanic Gardens in South Africa turned to females after being transplanted to a shadier site (Osborne, 1994). Two suckers removed from a known male plant of *E. senticosus* developed into female cone-producing plants (Steenkamp, 1995). A 40-year-old female *C. revoluta* in Japan produced a lateral branch bearing a male cone (Tomiyama, 1995). Norstog and Nicolls (1997) tell of a female *Zamia fisheri* that began producing male cones after being moved and repotted.

Figure 21-1. Clear evidence of a sex change: this garden specimen of *Encephalartos middelburgensis* produced a female cone and seed crop in 1990—as demonstrated by the seed remnants trapped in the stem apex—but then produced six male cones in 1991.

A particularly significant case of sex change occurred in a planting of *Cycas taitungensis* at the Brisbane International Airport, Australia, and was reported in *The Cycad Newsletter* (Osborne, 1998). Twelve mature male plants were purchased from a local nursery in July 1997; the nursery was selectively selling known male plants and keeping known females for seed production. The plants were placed in a site adjacent to the international arrivals hall in low-light (200–400 lux) environments, resulting in etiolated foliage and a general decline in appearance. Within six months, 11 of the 12 plants had produced cones—3 had produced female cones. In 1998, all plants were retransplanted to nearby site in bright sunlight (>1000 lux). The plants soon recovered, and there has been no evidence of any "double" sex change.

Recent reports of cycad sex changes in *Palms and Cycads* (Osborne & Gorelick, 2002) included those of a male *Ceratozamia matudae* moved from a glasshouse to a garden site in Los Angeles, U.S.A, in 1998, which coned as a male in 1999 but as a female in 2000 and 2001. A known male specimen of *E. senticosus*, shipped from South Africa to Los Angeles, produced leaf flushes for seven years and then a female cone. A pot-bound male *Zamia fairchildiana*, rescued from a particularly dry glasshouse in which it had not produced cones in 10 to 15 years, produced a male cone after being repotted and a female cone the following year. A large specimen of *Cycas thouarsii* in a garden in the Comores Islands produced cones as a male for eight to nine years but then changed to a female after it was physical damaged.

PREVIOUSLY UNRECORDED CASES OF SEX CHANGE IN CYCADS

Three new reports of cycad sex change have been brought to our attention. The first relates to a male specimen of *Lepidozamia peroffskyana*, planted in a low-light environment at the Mirage Casino in Las Vegas, U.S.A, which produced a male cone initially but then a female cone closely thereafter (D. Retzlaff, pers. comm.). The second is the history of a sucker removed from a well-established male specimen of *Encephalartos munchii* in a Brisbane, Australia, garden. This was removed from the parent in 1996, rooted, transplanted, but nearly destroyed by a weevil infestation in 2002. After the top section of the plant was rerooted, the apex produced three somewhat atrophied female cones in 2004 (S. Walkley, pers. comm.). The third and final case is that of a male *Zamia pygmaea* that produced a female cone after being moved and repotted (D. Stevenson, pers. comm.).

FALSE CASES OF SEX CHANGE IN CYCADS

There are several caveats to alleged cases of sex changes in cycads, and we have been careful to be cognizant of these. In our analysis, we have not included any purely anecdotal accounts that are unsupported by written or photographic evidence. Some cases of cycads "bearing both male and female cones" have proved to be two plants growing in very close proximity (Osborne & Gorelick, 2002). We are also are mindful of the phenomenon of two embryos developing from a single seed and giving what appears to be one plant with both male and female characteristics. All such dubious cases have been excluded from our analysis.

Discussion

The cases of sex reversal detailed in the preceding paragraphs are summarized in Table 21-1. Several significant points emerge from these data.

1. The recorded cases span 6 of the 11 cycad genera and 16 species.
2. All recorded cases are from plants growing in the artificial (garden) environment; there are no known cases of sex change from plants in the wild.

Table 21-1 Known cases of sex change in cycads.

Cycad species	Change	Circumstances	Reference
Ceratozamia matudae	M to F	Moved from glasshouse to garden	Osborne & Gorelick, 2002
Cycas circinalis (*C. rumphii* ?)	F to M	Bud from female	Chamberlain, 1935
C. circinalis (*C. rumphii* ?)	F to M	Mechanical damage	Menninger, 1967
C. circinalis (*C. rumphii* ?)	M to F	Severe cold	Menninger, 1967
Cycas revoluta	Unknown	Mechanical damage	Schuster, 1932
C. revoluta	F to M	Unknown	Chamberlain, 1935
C. revoluta	M to F	Transplanted	Kemp, 1985
C. revoluta	F to M	Unknown	Osborne, 1994
C. revoluta	F to M	Lateral branch	Tomiyama, 1995
Cycas taitungensis (3 plants)	M to F	Transplanted to low light environment	Osborne, 1998
Cycas thouarsii	M to F	Mechanical damage	Osborne & Gorelick, 2002
Encephalartos latifrons	F to M	Severe drought	Osborne, 1988
Encephalartos middelburgensis	F to M	Unknown	Osborne, 1991
Encephalartos munchii	M to F	Sucker from male	S.J. Walkley, pers. comm.
Encephalartos senticosus	F to M	Sucker from female	Osborne, 1990
E. senticosus (2 plants)	M to F	Suckers from male	Steenkamp, 1995
E. senticosus	M to F	Transplanted	Osborne & Gorelick, 2002
Encephalartos umbeluziensis	M to F	Severe cold	Van Wyk & Claassen, 1981
Encephalartos villosus	F to M	Severe drought	Osborne, 1990
E. villosus	F to M	Transplanting	Osborne, 1990
Lepidozamia peroffskyana	M to F	Transplanted to low light environment	D. Retzlaff, pers. comm.
Stangeria eriopus	F to M	Lateral branch	Anonymous, 1989
Zamia fairchildiana	M to F	Repotted and watering increased	Osborne & Gorelick, 2002
Zamia fischeri	F to M	Repotted and environment	Norstog & Nicholls, 1997
Zamia pygmaea	M to F	Repotted and environment changed	D. W. Stevenson, pers.comm.
Zamia vazquezii (4 plants)	M to F	Transplanted to a shadier site	Osborne, 1994
Zamia sp.	F to M	Side crown	Osborne, 1985

3. All recorded cases are unidirectional (i.e., there are no known incidents in which any cycad has changed sex and then undergone a reversal of the primary change).
4. Of the cases recorded, 19 are male-to-female and 13 are female-to-male; changes can therefore occur in either direction.
5. Male-to-female changes are associated with exposure to low-light environments in eight cases and exposure to severe cold in two cases.
6. Female-to-male changes are associated with severe drought in two cases.

A MECHANISM FOR SEX CHANGE IN CYCADS

We have presented the theoretical aspects of the biochemical process (i.e., the cellular chemistry of sex changes) in the *South African Journal of Science* (Gorelick & Osborne 2002). A simplified summary of our theory is given in the following two paragraphs.

Sexual development in plants and animals is governed by a suite of genes that act both synergistically and antagonistically to dictate the "maleness" or "femaleness" of an organism. There are separate (albeit related) genes that code for primary and secondary sexual characteristics. The DNA code is contained in a sequence of the four nucleotide bases (cytosine, guanine, adenine, thymine) that comprise the axis of each DNA strand. In the classic Watson–Crick model, two strands of DNA are spirally interwoven in a right-handed helix. This can be best envisioned as a rope ladder that is twisted to form a double helix. For a gene to encode a product (typically proteins), the double helix must have a steady, gentle right-handed twist.

Of the four nucleotides, cytosine is susceptible to a process known as methylation, in which the hydrogen atom (H) at the C-5 position is replaced by a methyl group (CH_3) (adenine can also be methylated, but not in nuclear genomes). Cytosine methylation permanently alters the nature of the DNA double helix and all "new" copies of the molecule will carry the same change (Holliday, 1988; Vyskot et al., 1995). Methyl groups are bulky and water repellant. This changes the steady, gentle right-handed twist of the DNA in two ways. First, imagine a rope ladder, with some securely attached some large beach balls, representing the methylation. The bulkiness of the beach balls grossly alters any twisting of the double helix. Second, keep in mind that cells are largely made of water, so that water-repellant molecules may be forced into different conformations from other molecules. There is even evidence that methylation processes can convert a right-handed DNA helix to a left-handed one (Behe & Felsenfeld, 1981; McKay & Steitz, 1981). Heterochromatic proteins also attach to cytosine methylation, which thereby occupy binding sites that otherwise could be occupied by those enzymes that mediate transcription of messenger RNA, which ultimately codes for production of gene products. Methylation of cytosine, especially in promoter regions of genes, effectively shuts down the gene (Fig. 21-2). Thus, cytosine methylation can cause quantitative and qualitative changes to the synthesis of various gene products, including sex hormones. More simply put, cytosine methylation can cause sex changes in dioecious plants.

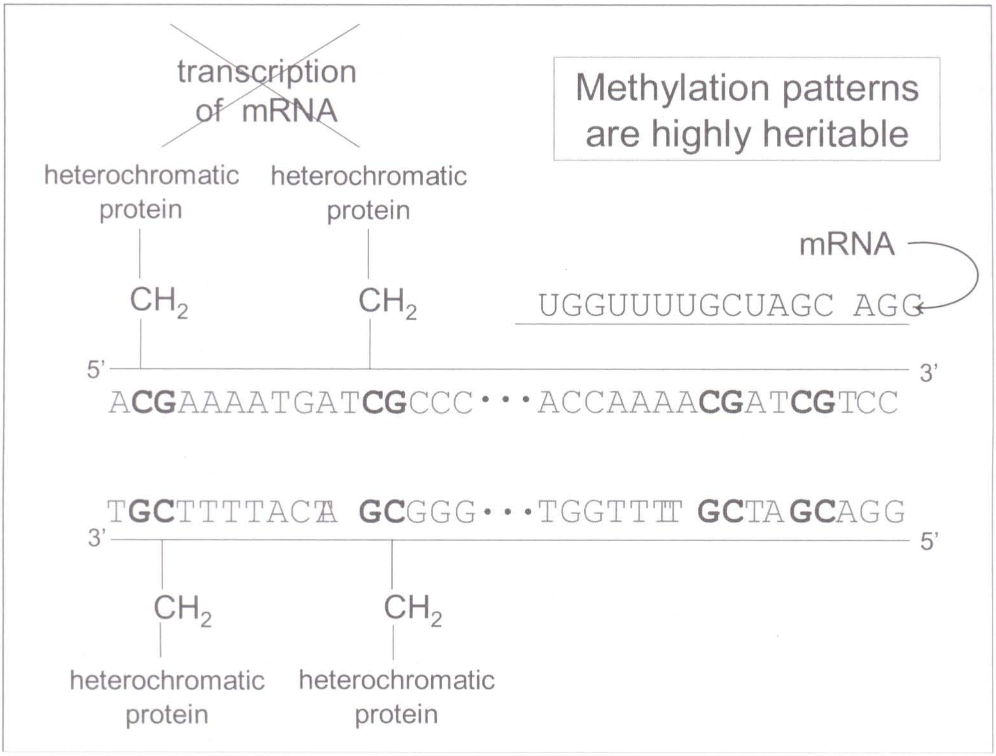

Figure 21-2. Methylation of cytosine shuts down genes. Methylated cytosine changes the confirmation of the double helix (not shown above), shutting down genes. Note that only cytosines in CG dinucleotides (or in CXG trinucleotides, where X can be A, C, G, or T) are usually methylated so as to maintain the methylation patterns following mitosis. Heterochromatic proteins bind to methylated cytosine, which thereby occupy binding sites that would otherwise be occupied by enzymes that mediate transcription. Gene activity is mediated by transcription of mRNA, which is then transported to the ribosomes where the mRNA signal gets converted (translated) into proteins. If, for example, the methylated gene was originally responsible for production of female gametes or sex hormones in a hermaphroditic lineage, then this methylated individual becomes a male.

Cytosine methylation can be removed by factors such as temperature (Dorazi et al., 1995; Demeulemeester et al., 1999), light (Tatra et al., 2000), and osmotic stress (Kovarich et al., 1997). The fact that variation in these environmental factors is associated with sex changes in cycads (as demonstrated in Table 21-1) is strongly supportive of the theory we have developed. Unidirectional sex change is consistent with this theory insofar because it is much simpler to remove methylation than to add it in a site-specific manner. We
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believe that most examples of sex change in cycads are probably due to removal of cytosine methylation as a consequence of environmental shock (Gorelick, 2003). Work is slowly progressing, although not with cycads, directly showing that methylation and other associated chromosomal changes turn genes for femaleness and maleness in plants on and off (Siljak-Yakovlev et al., 1996; Ma et al., 2004).

We believe that tissue culture techniques will allow researchers to manipulate methylation and demethylation artificially. We have already suggested that this avenue will ultimately permit induced sex changes in tissue from cycad species in which only one sex survives. In short, we have presented a potential recipe for the creation of a female *Encephalartos woodii*. By applying chemicals that effectively remove methylation, such as 5-azacytidine and ethionine, we believe it will be possible to change tissues from males of *E. woodii* and similarly endangered species into females or hermaphrodites.

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